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(57) Abstract

The present invention provides a recombinant α-L-iduronidase and biologically active fragments and mutants thereof, methods to produce and purify this enzyme as well as methods to treat certain genetic disorders including α-L-iduronidase deficiency and mucopolysaccharidosis I (MPS I).

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RECOMBINANT α-L-IDURONIDASE, METHODS FOR PRODUCING AND PURIFYING THE SAME AND METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES THEREOF

FIELD OF THE INVENTION

The present invention is in the field of molecular biology, enzymology, biochemistry and clinical medicine. In particular, the present invention provides a recombinant α-L-iduronidase, methods to produce and purify this enzyme as well as methods to treat certain genetic disorders including α-L-iduronidase deficiency and mucopolysaccharidosis I (MPS I).

BACKGROUND OF THE INVENTION

Carbohydrates play a number of important roles in the functioning of living organisms. In addition to their metabolic roles, carbohydrates are structural components of the human body covalently attached to numerous other entities such as proteins and lipids (called glycoconjugates). For example, human connective tissues and cell membranes comprise proteins, carbohydrates and a proteoglycan matrix. The carbohydrate portion of this proteoglycan matrix provides important properties to the body's structure.

A genetic deficiency of the carbohydrate-cleaving, lysosomal enzyme α-L-iduronidase causes a lysosomal storage disorder known as mucopolysaccharidosis I (MPS I) (Neufeld, E. F., and Muenzer, J. (1989). The mucopolysaccharidoses in "The Metabolic Basis of Inherited Disease" (Scriver, C.R., Beaudet, A. L., Sly, W. S., and Valle, D., Eds.), pp. 1565-1587, McGraw-Hill, New York). In a severe form, MPS I is commonly known as Hurler syndrome and is associated with multiple problems such as mental retardation, clouding of the cornea, coarsened facial features, cardiac disease, respiratory disease, liver and spleen enlargement, hernias, and joint stiffness. Patients suffering, from Hurler syndrome usually die before age 10. In an intermediate form known as Hurler-Scheie syndrome, mental function is generally not severely affected, but physical problems may lead to death by the teens or twenties. Scheie syndrome is the mildest form of MPS I. It is compatible with a normal life span, but joint stiffness, corneal clouding and heart valve disease cause significant problems.

The frequency of MPS I is estimated to be 1:100,000 according to a British Columbia survey of all newborns (Lowry *et al., Human Genetics* 85:389-390 (1990)) and 1:70,000 according to an Irish study (Nelson, *Human Genetics* 101:355-358 (1990)). There appears to be no ethnic predilection for this disease. It is likely that worldwide the disease is underdiagnosed either because the patient dies of a complication before the diagnosis is made or because the milder forms of the syndrome may be

mistaken for arthritis or missed entirely. Effective newborn screening for MPS I would likely find some previously undetected patients.

Except for bone marrow transplantation, there are no significant therapies available for MPS I. Bone marrow transplants can be effective in treating some of the symptoms of the disorder but have high morbidity and mortality in MPS I and often are not available to patients because of a lack of suitable donors. An alternative therapy available to all affected patients would provide an important breakthrough in treating and managing this disease.

Enzyme replacement therapy has long been considered a potential therapy for MPS I following the discovery that α-L-iduronidase can correct the enzymatic defect in Hurler cells in culture. In this corrective process, the enzyme containing a mannose-6-phosphate residue is taken up into cells through receptor-mediated endocytosis and transported to the lysosomes where it clears the stored substrates, heparan sulfate and dermatan sulfate. Application of this therapy to humans has previously not been possible due to inadequate sources of α-L-iduronidase in tissues. The enzyme replacement concept was first effectively applied to Gaucher patients in a modified placental glucocerebrosidase. The delivery and effective uptake of glucocerebrosidase in Gaucher patients demonstrated that an enzyme could be taken up *in vivo* in sufficient quantities to provide effective therapy.

For α-L-iduronidase enzyme therapy in MPS I, a recombinant source of enzyme has been needed in order to obtain therapeutically sufficient supplies of the enzyme. The mammalian enzyme was cloned in 1990 (Stoltzfus *et al., J. Biol. Chem.* 267:6570-6575 (1992), and the human enzyme was cloned in 1991 (Moskowitz *et al., FASEB J* 6:A77 (1992)).

DESCRIPTION OF THE FIGURES

FIGURE 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding α-L-iduronidase. Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.

FIGURE 2 represents the results from an SDS-PAGE run of eluate obtained according to the procedure set forth in Example 1. Lane 1 is blank. Lane 2 contained high molecular weight standards. Lane 3 is a blank. Lane 4 contained bovine serum albumin in a concentration of 50 μg. Lanes 5 through 10 represent eluate containing recombinantly produced human α-L-iduronidase in amounts of 1 μg, 2 μg, 5 μg, 5 μg, 5 μg and 5 μg, respectively.

FIGURE 3 reveals the urinary GAG levels in 16 MPS I patients in relation to normal excretion values. There is a wide range of urine GAG values in untreated MPS I patients. A greater than 50%

reduction in excretion of undegraded GAGs following therapy with recombinant α-L-iduronidase is a valid means to measure an individual's response to therapy.

FIGURE 4 demonstrates leukocyte iduronidase activity before and after enzyme therapy in MPS I patients.

FIGURE 5 demonstrates the buccal iduronidase activity before and after enzyme therapy.

FIGURE 6 demonstrates in three patients that a substantial shrinkage of liver and spleen together with significant clinical improvement in joint and soft tissue storage was associated with a greater than 65% reduction in undegraded GAG after only 8 weeks of treatment with recombinant enzyme.

FIGURE 7 demonstrates that there is substantial normalization of livers and spleens in patients treated with recombinant enzyme after only 12 weeks of therapy.

FIGURE 8 demonstrates a precipitous drop in urinary GAG excretion over 22 weeks of therapy with recombinant enzyme in 6 patients.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention features a method to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. In a broad embodiment, the method comprises the step of transfecting a cDNA encoding for all or a part of an α-L-iduronidase into a cell suitable for the expression thereof. In some embodiments, a cDNA encoding for a complete α-L-iduronidase is used, preferably a human α-L-iduronidase. However, in other embodiments, a cDNA encoding for a biologically active fragment or mutant thereof may be used. Specifically, one or more amino acid substitutions may be made while preserving or enhancing the biological activity of the enzyme. In other preferred embodiments, an expression vector is used to transfer the cDNA into a suitable cell or cell line for expression thereof. In one particularly preferred embodiment, the cDNA is transfected into a Chinese hamster ovary cell to create cell line 2.131. In yet other preferred embodiments, the production procedure features one or more of the following characteristics which have demonstrated particularly high production levels: (a) the pH of the cell growth culture may be lowered to about 6.5 to 7.0, preferably to about 6.7-6.8 during the production process, (b) about 2/3 to 3/4 of the medium may be changed approximately every 12 hours, (c) oxygen saturation may be optimized at about 80% using intermittent pure oxygen sparging, (d) microcarriers with about 10% serum initially may be used to produce cell mass followed by a rapid washout shift to protein-free medium for production, (e) a protein-free or low protein medium such as a JRH Biosciences PF-CHO product may be optimized to include supplemental amounts of one or more ingredients selected from the group consisting of glutamate, aspartate, glycine, ribonucleosides and deoxyribonucleosides, (f) a perfusion

wand such as a Bellco perfusion wand may be used in a frequent batch-feed process rather than a standard intended perfusion process, and (g) a mild sodium butyrate induction process may be used to induce increased α-L-iduronidase expression.

In a second aspect, the present invention provides a transfected cell line which features the ability to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. In preferred embodiments, the present invention features a recombinant Chinese hamster ovary cell line such as the 2.131 cell line that stably and reliably produces amounts of α-L-iduronidase which enable using the enzyme therapeutically. In some preferred embodiments, the cell line may contain at least about 10 copies of a an expression construct. In even more preferred embodiments, the cell line expresses recombinant α-L-iduronidase in amounts of at least about 20-40 micrograms per 10^7 cells per day.

In a third aspect, the present invention provides novel vectors suitable to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. In preferred embodiments, the present invention features an expression vector comprising a cytomegalovirus promoter/enhancer element, a 5' intron consisting of a murine Ca intron, a cDNA encoding all or a fragment or mutant of an α-L-iduronidase, and a 3' bovine growth hormone polyadenylation site. Also, preferably the cDNA encoding all or a fragment or mutant of an α-L-iduronidase is about 2.2 kb in length. This expression vector may be transfected at, for example, a 50 to 1 ratio with any appropriate common selection vector such as, for example, pSV2NEO, to enhance multiple copy insertions. Alternatively, gene amplification may be used to induce multiple copy insertions.

In a fourth aspect, the present invention provides novel α-L-iduronidase produced in accordance with the methods of the present invention and thereby present in amounts which enable using the enzyme therapeutically. The specific activity of the α-L-iduronidase according to the present invention is in excess of 200,000 units per milligram protein. Preferably, it is in excess of about 240,000 units per milligram protein. The molecular weight of the α-L-iduronidase of the present invention is about 82,000 daltons, about 70,000 daltons being amino acid and about 12,000 daltons being carbohydrates.

In a fifth aspect, the present invention features a novel method to purify α-L-iduronidase. According to a first embodiment, a cell mass may be grown in about 10% serum containing medium followed by a switch to a modified protein-free production medium without any significant adaptation to produce a high specific activity starting material for purification. Preferably, a concentration/diafiltration scheme is employed that allows for the removal of exogenous materials that may be required for recombinant production of the same such as, for example, Pluronics F-68, a

commonly used surfactant for protecting cells from sparging damage. Such exogenous materials should normally be separated from the crude bulk to prevent fouling of the columns. In another preferred embodiment, a first column load is acidified to minimize the competitive inhibition effect of uronic acids found in protein-free medium formulations. Also preferably, a heparin, phenyl and sizing column purification scheme is used to produce pure enzyme using automatable steps and validatable media. In another preferred embodiment, the heparin and phenyl column steps are used to eliminate less desirable α-L-iduronidase that is nicked or degraded. In another preferred embodiment, an acid pH treatment step is used to inactivate potential viruses without harming the enzyme.

In a sixth aspect, the present invention features novel methods of treating diseases caused all or in part by a deficiency in α-L-iduronidase. In one embodiment, this method features administering a recombinant α-L-iduronidase or a biologically active fragment or mutant thereof alone or in combination with a pharmaceutically suitable carrier. In other embodiments, this method features transferring a nucleic acid encoding all or a part of an α-L-iduronidase into one or more host cells *in vivo*. Preferred embodiments include optimizing the dosage to the needs of the organism to be treated, preferably mammals or humans, to effectively ameliorate the disease symptoms. In preferred embodiments, the disease is mucopolysaccharidosis I (MPS I), Hurler syndrome, Hurler-Scheie syndrome or Scheie syndrome.

In a seventh aspect, the present invention features novel pharmaceutical compositions comprising α-L-iduronidase useful for treating a disease caused all or in part by a deficiency in α-L-iduronidase. Such compositions may be suitable for administration in a number of ways such as parenteral, topical, intranasal, inhalation or oral administration. Within the scope of this aspect are embodiments featuring nucleic acid sequences encoding all or a part of an α-L-iduronidase which may be administered *in vivo* into cells affected with an α-L-iduronidase deficiency.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention features a method to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. In general, the method features transforming a suitable cell line with the cDNA encoding for all of α-L-iduronidase or a biologically active fragment or mutant thereof. Those of skill in the art may prepare expression constructs other than those expressly described herein for optimal production of α-L-iduronidase in suitable cell lines transfected therewith. Moreover, skilled artisans may easily design fragments of cDNA encoding biologically active fragments and mutants of the naturally occurring α-L-iduronidase which possess the same or similar biological activity to the naturally occurring full-length enzyme.

To create a recombinant source for α-L-iduronidase, a large series of expression vectors may be constructed and tested for expression of a α-L-iduronidase cDNA. Based on transient transfection experiments as well as stable transfections, an expression construct may be identified that provides particularly high level expression. In one embodiment of the present invention, a Chinese hamster cell line 2.131 developed by transfection of the α-L-iduronidase expression construct and selection for a high expression clone provides particularly high level expression. Such a Chinese hamster cell line according to this embodiment of the present invention may secrete about 5,000 to 7,000 fold more α-L-iduronidase than normal. The α-L-iduronidase produced thereby may be properly processed, taken up into cells with high affinity and is corrective for α-L-iduronidase deficient cells such as those from patients suffering from Hurler's Syndrome.

The method for producing α-L-iduronidase in amounts that enable using the enzyme therapeutically features a production process specifically designed to produce the enzyme in high quantities. According to preferred embodiments of such a process, microcarriers are used as a low cost scalable surface on which to grow adherent cells.

According to other preferred embodiments of the method for producing α-L-iduronidase according to the present invention, a culture system is optimized. In a first embodiment, the culture pH is lowered to about 6.5 to 7.0, preferably to about 6.7-6.8 during the production process. One advantage of such a pH is to enhance accumulation of lysosomal enzymes that are more stable at acidic pH. In a second embodiment, about 2/3 to 3/4 of the medium is changed approximately every 12 hours. One advantage of this procedure is to enhance the secretion rate of recombinant α-L-iduronidase and capture more active enzyme. In a third embodiment, oxygen saturation is optimized at about 80% using intermittent pure oxygen sparging rather than continuous sparging. In a fourth embodiment, cytodex 2 microcarriers with about 10% serum initially are used to produce a cell mass followed by a rapid washout shift to a protein-free medium for production. In a fifth embodiment, a growth medium such as a JRH Biosciences PF-CHO product may be optimized to include supplemental amounts of one or more ingredients selected from the group consisting of glutamate, aspartate, glycine, ribonucleosides and deoxyribonucleosides. In a sixth embodiment, a perfusion wand such as a Bellco perfusion wand may be used in a frequent batch-feed process rather than a standard intended perfusion process. In a seventh embodiment, a mild sodium butyrate induction process may be used to induce increased α-L-iduronidase expression without a substantial effect on the carbohydrate processing and cellular uptake of the enzyme. Such an induction process may provide about a two-fold increase in production without significantly altering post-translational processing.

Particularly preferred embodiments of the method for producing α-L-iduronidase according to the present invention feature one, more than one or all of the optimizations described herein. The production method of the present invention may therefore provide a production culture process having the following features:

1. A microcarrier based culture using Cytodex 2 beads or an equivalent thereof is preferably used in large scale culture flasks with overhead wand stirring using a Bellco perfusion wand or an equivalent thereof. Attachment to these beads may be achieved by culture in a 10% fetal bovine serum in DME/F12 1:1 medium modified with ingredients including ribonucleosides, deoxyribonucleosides, pyruvate, non-essential amino acids, and HEPES and at a pH of about 6.7-6.9. After about 3 days in this medium, a washout procedure is begun in which protein-free medium replaces approximately 2/3 of the medium approximately every 12 hours for a total of about 3-4 washes. Subsequently and throughout the entire remaining culture period, the cells are cultivated in protein-free medium.

2. The culture conditions are preferably maintained at a dissolved oxygen of 80% of air saturation at a pH of about 6.7 and at a temperature of about 37° C. This may be achieved using a control tower, service unit and appropriate probes such as those produced by Wheaton. However, skilled artisans will readily appreciate that this can easily be achieved by equivalent control systems produced by other manufacturers. An air saturation of about 80% results in improved α-L-iduronidase secretion over 40% and 60% air saturation. However 90% air saturation does not provide significantly enhanced secretion over 80% air saturation. The dissolved oxygen may be supplied by intermittent pure oxygen sparging using a 5 micron stainless steel sparger or equivalent thereof. A pH of about 6.7 is optimal for the accumulation of the α-L-iduronidase enzyme. The enzyme is particularly unstable at pH's above about 7.0. Below a pH of about 6.7, the secretion rate may decrease, particularly below a pH of about 6.5. The culture is therefore maintained optimally between a pH of about 6.6 to 6.8 .

3. The production culture medium may be a modified form of the commercially available proprietary medium from JRH Biosciences called Excell PF CHO. This medium supports levels of secretion equivalent to that of serum using a cell line such as the 2.131 cell line. It may be preferably modified to include an acidic pH of about 6.7 (+/- 0. 1), and it may be buffered with HEPES at 7.5 mM. The medium may contain 0.05 to 0.1% of Pluronics F-68 (BASF), a non-ionic surfactant or an equivalent thereof which features the advantage of protecting cells from shear forces associated with sparging. The medium may further contain a proprietary supplement that proves to be important in increasing the productivity of the medium over other protein-free mediums that are presently available. Those skilled in the art will readily understand that the choice of culture medium

may be optimized continually according to particular commercial embodiments available at particular points in time. Such changes encompass no more than routine experimentation and are intended to be within the scope of the present invention.

4. The production medium may be analyzed using an amino acid analyzer comparing spent medium with starting medium. Such analyses have demonstrated that the 2.131 cell line depletes a standard PF CHO medium of glycine, glutamate and aspartate to a level of around 10% of the starting concentration. Supplementation of these amino acids to higher levels may result in enhanced culture density and productivity that may lead to a 2-3 fold higher production than at baseline. Skilled artisans will appreciate that other cell lines within the scope of the present invention may be equally useful for producing α-L-iduronidase according to the present method. Hence, more or less supplemental nutrients may be required to optimize the medium. Such optimizations are intended to be within the scope of the present invention and may be practiced without undue experimentation.

5. The medium may be supplemented with ribonucleosides and deoxyribonucleosides to support the dihydrofolate reductase deficient cell line 2.131. Skilled artisans will appreciate that other cell lines within the scope of the present invention may be equally useful for producing α-L-iduronidase according to the present method. Hence, more or less ribonucleosides and deoxyribonucleosides may be required to optimize the medium. Such optimizations are intended within the scope of the present invention and may be practiced without undue experimentation.

6. After reaching confluence at about 3-4 days of culture, approximately 2/3 of the medium may be changed out approximately every 12 hours. The change out of medium may be accomplished using, for instance, a Bellco perfusion wand which is a stirring device with a hollow center and screen filter at its tip. By pumping out medium through the hollow interior of the wand through the 40 micron screen. The microcarriers with the 2.131 cell mass are separated from supernatant containing the enzyme.

7. The rapid and frequent turnover of the medium has been shown by productivity studies to result in improved overall collection of enzyme from the cell culture. Less frequent changes result in less total accumulation of enzyme. Studies of the secretion rate of the enzyme during a 12 hour culture cycle demonstrate that the cells are actively secreting enzyme for the majority of the culture period. More frequent changes are unlikely to yield substantially more enzyme. The method of this embodiment has proven to be superior to perfusion culture and far superior to strict batch culture or daily or every other day batch/feed strategies. Using the every approximately 12 hour change, the cells may be maintained in excellent condition with high degrees of viability and a high level of productivity.

8. Production of α-L-iduronidase may be enhanced by the use of sodium butyrate induction of gene expression. Systematic studies of a 2.131 cell line demonstrated that about 2 mM butyrate can be applied and result in about a two-fold or greater induction of enzyme production with minimal effects on carbohydrate processing. Lower levels of butyrate have not been shown to induce as well, and substantially higher levels may result in higher induction but declining affinity of the produced enzyme for cells from patients suffering from α-L-iduronidase deficiency. The results suggest that two-fold or greater induction results in less processing of the carbohydrates and less phosphate addition to the enzyme as well as increasing toxicity. One particularly preferred method uses 2 mM butyrate addition every 48 hours to the culture system. This embodiment results in about a two-fold induction of enzyme production using this method without significant effect on the uptake affinity of the enzyme, (K-uptake of less than 30 U/ml or 2.0 mM). Using embodiments of the present method featuring all of the above modifications and induction, a 15 liter culture system may produce approximately 25 mg per liter of culture per day, or more at peak culturing density.

In a second aspect, the present invention provides a transfected cell line which possesses the unique ability to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. In preferred embodiments, the present invention features a recombinant Chinese hamster ovary cell line such as the 2.131 cell line that stably and reliably produces amounts of α-L-iduronidase. In preferred embodiments, the cell line may contain at least about 10 copies of an expression construct comprising a CMV promoter, a Ca intron, a human α-L-iduronidase cDNA, and a bovine growth hormone polyadenylation sequence. In even more preferred embodiments, the cell line expresses α-L-iduronidase at amounts of at least about 20-40 micrograms per 10^7 cells per day in a properly processed, high uptake form appropriate for enzyme replacement therapy. According, to preferred embodiments of this aspect of the invention, the transfected cell line adapted to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically possesses one or more of the following features:

1. The cell line of preferred embodiments is derived from a parent cell line wherein the cells are passaged in culture until they have acquired a smaller size and more rapid growth rate and until they readily attach to substrates.

2. The cell line of preferred embodiments is transfected with an expression vector containing the 2 and 3, a human cDNA of about 2.2 kb in length, and a 3' bovine growth hormone cytomegalovirus promoter/enhancer element, a 5' intron consisting of the murine Ca intron between exons polyadenylation site. This expression vector may be transfected at, for example, a 50 to 1 ratio with any appropriate common selection vector such as pSV2NEO. The selection vector pSV2NEO in

turn confers G418 resistance on successfully transfected cells. In particularly preferred embodiments, a ratio of about 50 to 1 is used since this ratio enhances the acquisition of multiple copy number inserts. According to one embodiment wherein the Chinese hamster ovary cell line 2.131 is provided, there are approximately 10 copies of the expression vector for α-L-iduronidase. Such a cell line has demonstrated the ability to produce large quantities of human α-L-iduronidase (minimum 20 micrograms per 10 million cells per day). Particularly preferred embodiments such as the 2.131 cell line possess the ability to produce properly processed enzyme that contains N-linked oligosaccharides containing high mannose chains modified with phosphate at the 6 position in sufficient quantity to produce an enzyme with high affinity (K-uptake of less than 3 nM).

3. The enzyme produced from the cell lines of the present invention such as a Chinese hamster ovary cell line 2.131 is rapidly assimilated into cells, eliminates glycosaminoglycan storage and has a half-life of about 5 days in cells from patients suffering from α-L-iduronidase deficiency.

4. The cell line of preferred embodiments such as a 2.131 cell line adapts to large scale culture and stably produces human α-L-iduronidase under these conditions. The cells of preferred embodiments are able to grow and secrete α-L-iduronidase at the acid pH of about 6.6 to 6.8 at which enhanced accumulation of α-L-iduronidase can occur.

5. Particularly preferred embodiments of the cell line according to the invention, such as a 2.131 cell line are able to secrete human α-L-iduronidase at levels exceeding 2,000 units per ml (8 micrograms per ml) twice per day using a specially formulated protein-free medium.

In a third aspect, the present invention provides novel vectors suitable to produce α-L-iduronidase in amounts which enable using the enzyme therapeutically. The production of adequate quantities of recombinant α-L-iduronidase is a critical prerequisite for studies on the structure of the enzyme as well as for enzyme replacement therapy. The cell lines according to the present invention permit the production of significant quantities of recombinant α-L-iduronidase that is appropriately processed for uptake. Overexpression in Chinese hamster ovary (CHO) cells has been described for three other lysosomal enzymes, α-galactosidase (Ioannou *et al., J Cell. Biol.* 119:1137-1150 (1992)), iduronate 2-sulfatase (Bielicki *et al., Biochem. J.* 289: 241-246 (1993)), and N-acetylgalactosamine 4 -sulfatase (Anson *et al., Biochem. J.* 284:789-794 (1992)), using, a variety of promoters and, in one case, amplification. The present invention features a dihydrofolate reductase-deficient CHO cell line, but according to preferred embodiments of the invention amplification is unnecessary. Additionally, the present invention provides a high level of expression of the human α-L-iduronidase using the CMV immediate early gene promoter/enhancer.

The present invention features in preferred embodiments an expression vector comprising a cytomegalovirus promoter/enhancer element, a 5' intron consisting of the murine Ca intron derived from the murine long chain immunoglobulin Cα gene between exons 2 and 3, a human cDNA of about 2.2 kb in length, and a 3' bovine growth hormone polyadenylation site. This expression vector may be transfected at, for example, a 50 to 1 ratio with any appropriate common selection vector such as, for example, pSV2NEO. The selection vector such as pSV2NEO in turn confers G418 resistance on successfully transfected cells. In particularly preferred embodiments, a ratio of about 50 to 1 expression vector to selection vector is used since this ratio enhances the acquisition of multiple copy number inserts. According to one embodiment wherein the Chinese hamster ovary cell line 2.131 is provided, there are approximately 10 copies of the expression vector for α-L-iduronidase. Such an expression construct has demonstrated the ability to produce large quantities of human α-L-iduronidase (minimum 20 micrograms per 10 million cells per day) in a suitable cell line such as, for example, a Chinese hamster ovary cell line 2.131.

In a fourth aspect, the present invention provides novel α-L-iduronidase produced in accordance with the methods of the present invention and thereby present in amounts that enable using the enzyme therapeutically. The methods of the present invention produce a substantially pure α-L-iduronidase that is properly processed and in high uptake form appropriate for enzyme replacement therapy and that is effective in therapy *in vivo*.

The specific activity of the α-L-iduronidase according to the present invention is in excess of about 200,000 units per milligram protein. Preferably, it is in excess of about 240,000 units per milligram protein. The molecular weight of the full length α-L-iduronidase of the present invention is about 82,000 daltons comprising about 70,000 daltons of amino acids and 12,000 daltons of carbohydrates. The recombinant enzyme of the present invention is endocytosed even more efficiently than has been previously reported for a partially purified preparation of urinary enzyme. The recombinant enzyme according to the present invention is effective in reducing the accumulation of radioactive S-labeled GAG in α-L-iduronidase-deficient fibroblasts, indicating that it is transported to lysosomes, the site of GAG storage. The remarkably low concentration of α-L-iduronidase needed for such correction (half-maximal correction at 0.7 pM) may be very important for the success of enzyme replacement therapy.

The human cDNA of α-L-iduronidase predicts a protein of 653 amino acids and an expected molecular weight of 70,000 daltons after signal peptide cleavage. Amino acid sequencing reveals alanine 26 at the N-terminus giving an expected protein of 629 amino acids. Human recombinant α-L-iduronidase has a Histidine at position 8 of the mature protein. The predicted protein sequence

comprises six potential N-linked oligosaccharide modification sites. All of these may be modified in the recombinant protein. The third and sixth sites have been demonstrated to contain one or more mannose 6-phosphate residues responsible for high affinity uptake into cells. The following peptide corresponds to Amino Acids 26-45 of Human Recombinant α-L-iduronidase with an N-terminus alanine and the following sequence:

ala-glu-ala-pro-his-leu-val-his-val-asp-ala-ala-arg-ala-leu-trp-pro-leu-arg-arg

The overexpression of the α-L-iduronidase of the present invention does not result in generalized secretion of other lysosomal enzymes that are dependent on mannose-6-P targeting. The secreted recombinant α-L-iduronidase is similar to normal secreted enzyme in many respects. Its molecular size, found in various determinations to be 77, 82, 84, and 89 kDa, is comparable to 87 kDa, found for urinary corrective factor (Barton *et al., J. Biol. Chem.* 246: 7773-7779 (1971)), and to 76 kDa and 82 kDa, found for enzyme secreted by cultured human fibroblasts (Myerowitz *et al., J. Biol. Chem.* 256: 3044-3048 (1991); Taylor *et al., Biochem. J* 274:263-268 (1991)). The differences within and between the studies are attributed to imprecision of the measurements. The pattern of intracellular processing of the recombinant enzyme-a slow decrease in molecular size and the eventual appearance of an additional band smaller by 9 kDa is the same as for the human fibroblast enzyme. This faster band arises by proteolytic cleavage of 80 N-terminal amino acids .

In a fifth aspect, the present invention features a novel method to purify α-L-iduronidase. In preferred embodiments, the present invention features a method to purify recombinant α-L-iduronidase that has been optimized to produce a rapid and efficient purification with validatible chromatography resins and easy load, wash and elute operation. The method of purifying α-L-iduronidase of the present invention involves a series of column chromatography steps which allow the high yield purification of enzyme from protein-free production medium.

According to a first embodiment, the cell mass is grown in about 10 % serum containing medium followed by a switch to a modified protein-free production medium without any significant adaptation to produce a high specific activity starting material for purification. In a second embodiment, a concentration/diafiltration scheme is employed that allows for the removal of such exogenous materials as Pluronics F-68 from the crude bulk to prevent fouling of columns. In a third embodiment, a first column load is acidified to minimize the competitive inhibition effect of such compounds as uronic acids found in protein-free medium formulations. In a fourth embodiment, a heparin, phenyl and sizing column purification scheme is used to produce pure enzyme using automatable steps. In a fifth embodiment, the heparin and phenyl column steps are used to eliminate

less desirable α-L-iduronidase that is nicked or degraded. In a sixth embodiment, an acid pH treatment step is used to inactivate potential viruses without harming the enzyme.

Particularly preferred embodiments of the method for purifying α-L-iduronidase according to the present invention feature more than one or all of the optimizations according to the following particular embodiments. The purification method of the present invention may therefore provide a purified α-L-iduronidase having the characteristics described herein.

1. Concentration/diafiltration: Crude supernatant is processed with a hollow fiber concentrator (A/G Technologies, 30K cutoff) to reduce fluid volume by about 75% and is then diafiltrated with a heparin load buffer (10 mM $NaPO_4$, pH 5.3, NaCl 200 mM). The diafiltration is an important step that eliminates undesirable compounds such as Pluronics F-68 from the supernatant, a surfactant needed in many cell cultures of the present invention that can foul columns. The diafiltration may also partly remove competitor inhibitors that may prevent binding to the heparin column. These inhibitors may be found in PF-CHO medium and are believed to be uronic acids derived from a soybean hydrolysate present in this particular medium.

2. Heparin column: The load may be adjusted to a pH of about 5.0 before loading on Heparin Sepharose CL-6B. Other types of heparin columns such as a heparin FF (Pharmacia) have different linkages and do not bind α-L-iduronidase as efficiently. A lower pH neutralizes uronic acids to some extent which lessens their competitive effect. Without the diafiltration and pH adjustment, heparin columns cannot be run using PF-CHO medium without having substantial enzyme flowthrough. The column may be washed with a pH of about 5.3 buffer and then eluted in 0.6 M NaCl. The narrow range of binding and elution salt concentration leads to an efficient purification step and enzyme that is often greater than 90% pure after one step.

3. Phenyl column: A Phenyl-Sepharose BP (Pharmacia) may be used in the next step. The heparin eluate may be adjusted to about 1.5 M NaCl and loaded on the column. The choice of resin is important as is the salt concentration in ensuring that the enzyme binds completely (no flow through) and yet elutes easily and completely with about 0.15 M NaCl. The eluate obtained is nearly pure α-L-iduronidase.

4. A pH inactivation may be performed to provide a robust step for the removal of potential viruses. The phenyl pool is adjusted to a pH of about 3.3 using Citrate pH 3.0 and held at room temperature for about 4 hours. The enzyme may then be neutralized. Embodiments featuring this step have been shown to eliminate viruses at a minimum of about 5 log units. The step does not substantially inactivate or affect the enzyme activity.

5. The enzyme may then be concentrated and injected onto a Sephacryl S-200 column and the peak of enzyme collected.

Enzyme purified in this manner has been shown to contain mannose-6-phosphate residues of sufficient quantity at positions 3 and 6 of the N-linked sugars to give the enzyme uptake affinity of less than 30 units per ml (less than 2 nM) enzyme. The enzyme is substantially corrective for glycosamino glycan storage disorders and has a half-life inside cells of approximately 5 days.

In a sixth aspect, the present invention features novel methods of treating diseases caused all or in part by a deficiency in α-L-iduronidase. Recombinant α-L-iduronidase provides enzyme replacement therapy in a canine model of MPS 1. This canine model is deficient in α-L-iduronidase due to a genetic mutation and is similar to human MPS 1. Purified, properly processed α-L-iduronidase was administered intravenously to 11 dogs. In those dogs treated with weekly doses of 25,000 to 125,000 units per kg for 3, 6 or 13 months, the enzyme was taken up in a variety of tissues and decreased the lysosomal storage in many tissues. The long term treatment of the disease was associated with clinical improvement in demeanor, joint stiffness, coat and growth. Higher doses of therapy (125,000 units per kg per week) result in better efficacy and including normalization of urinary GAG excretion in addition to more rapid clinical improvement in demeanor, joint stiffness and coat.

Enzyme therapy at even small doses of 25,000 units (0.1 mg/kg/wk) resulted in significant enzyme distribution to some tissues and decreases in GAG storage. If continued for over 1 year, significant clinical effects of the therapy were evident in terms of activity, mobility, growth and overall health. The therapy at this dose did not improve other tissues that are important sites for disease in this entity such as cartilage and brain. Higher doses of 125,000 units (0.5 mg/kg) given 5 times over two weeks demonstrate that improved tissue penetration can be achieved, and a therapeutic effect at the tissue level was accomplished in as little as 2 weeks. Studies at this increased dose haven been preformed in two dogs. These MPS I dogs show significant clinical improvement and substantial decreases in urinary GAG excretion into the normal range. Other than an immune reaction controlled by altered administration techniques, the enzyme therapy has not shown significant clinical or biochemical toxicity. Enzyme therapy at this higher weekly dose is effective at improving some clinical features of MPS I and decreasing storage without significant toxicity.

In a seventh aspect, the present invention features novel pharmaceutical compositions comprising human α-L-iduronidase useful for treating a deficiency in α-L-iduronidase. The recombinant enzyme may be administered in a number of ways such as parenteral, topical, intranasal, inhalation or oral administration. Another aspect of the invention is to provide for the administration of the enzyme by formulating it with a pharmaceutically-acceptable carrier which may be solid, semi-solid or liquid or an ingestable capsule. Examples of pharmaceutical compositions include tablets, drops such as nasal drops, compositions for topical application such as ointments, jellies, creams and

suspensions, aerosols for inhalation, nasal spray, and liposomes. Usually the recombinant enzyme comprises between 0.05 and 99% or between 0.5 and 99% by weight of the composition, for example, between 0.5 and 20% for compositions intended for injection and between 0.1 and 50% for compositions intended for oral administration.

To produce pharmaceutical compositions in this form of dosage units for oral application containing a therapeutic enzyme, the enzyme may be mixed with a solid, pulverulent carrier, for example lactose, saccharose, sorbitol, mannitol, a starch such as potato starch, corn starch, amylopectin, laminaria powder or citrus pulp powder, a cellulose derivative or gelatine and also may include lubricants such as magnesium or calcium stearate or a Carbowax or other polyethylene glycol waxes and compressed to form tablets or cores for dragees. If dragees are required, the cores may be coated for example with concentrated sugar solutions which may contain gum arabic, talc and/or titanium dioxide, or alternatively with a film forming agent dissolved in easily volatile organic solvents or mixtures of organic solvents. Dyestuffs can be added to these coatings, for example, to distinguish between different contents of active substance. For the composition of soft gelatine capsules consisting of gelatine and, for example, glycerol as a plasticizer, or similar closed capsules, the active substance may be admixed with a Carbowax® or a suitable oil as *e.g.,* sesame oil, olive oil, or arachis oil. Hard gelatine capsules may contain granulates of the active substance with solid, pulverulent carriers such as lactose, saccharose, sorbitol, mannitol, starches such as potato starch, corn starch or amylopectin, cellulose derivatives or gelatine, and may also include magnesium stearate or stearic acid as lubricants.

Therapeutic enzymes of the subject invention may also be administered parenterally such as by subcutaneous, intramuscular or intravenous injection or by sustained release subcutaneous implant. In subcutaneous, intramuscular and intravenous injection, the therapeutic enzyme (the active ingredient) may be dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material may be suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cottonseed oil and the like. Other parenteral vehicles such as organic compositions using solketal, glycerol, formal, and aqueous parenteral formulations may also be used.

For parenteral application by injection, compositions may comprise an aqueous solution of a water soluble pharmaceutically acceptable salt of the active acids according to the invention, desirably in a concentration of 0.5-10%, and optionally also a stabilizing agent and/or buffer substances in aqueous solution. Dosage units of the solution may advantageously be enclosed in ampules.

When therapeutic enzymes are administered in the form of a subcutaneous implant, the compound is suspended or dissolved in a slowly dispersed material known to those skilled in the art,

or administered in a device which slowly releases the active material through the use of a constant driving force such as an osmotic pump. In such cases, administration over an extended period of time is possible.

For topical application, the pharmaceutical compositions are suitably in the form of an ointment, gel, suspension, cream or the like. The amount of active substance may vary, for example, between 0.05- 20% by weight of the active substance. Such pharmaceutical compositions for topical application may be prepared in known manner by mixing the active substance with known carrier materials such as isopropanol, glycerol, paraffin, stearyl alcohol, polyethylene glycol, etc. The pharmaceutically acceptable carrier may also include a known chemical absorption promoter. Examples of absorption promoters are, *e.g.*, dimethylacetamide (U.S. Patent No. 3,472,931), trichloro ethanol or trifluoroethanol (U.S. Patent No. 3,891,757), certain alcohols and mixtures thereof (British Patent No. 1,001,949). A carrier material for topical application to unbroken skin is also described in the British patent specification No. 1,464,975, which discloses a carrier material consisting of a solvent comprising 40-70% (v/v) isopropanol and 0-60% (v/v) glycerol, the balance, if any, being an inert constituent of a diluent not exceeding 40% of the total volume of solvent.

The dosage at which the therapeutic enzyme containing pharmaceutical compositions are administered may vary within a wide range and will depend on various factors such as, for example, the severity of the disease, the age of the patient, etc., and may have to be individually adjusted. As a possible range for the amount of therapeutic enzyme which may be administered per day be mentioned from about 0.1 mg to about 2000 mg or from about 1 mg to about 2000 mg.

The pharmaceutical compositions containing the therapeutic enzyme may suitably be formulated so that they provide doses within these ranges either as single dosage units or as multiple dosage units. In addition to containing a therapeutic enzyme (or therapeutic enzymes), the subject formulations may contain one or more substrates or cofactors for the reaction catalyzed by the therapeutic enzyme in the compositions. Therapeutic enzyme containing compositions may also contain more than one therapeutic enzyme.

The recombinant enzyme employed in the subject methods and compositions may also be administered by means of transforming patient cells with nucleic acids encoding the recombinant α-L-iduronidase. The nucleic acid sequence so encoding may be incorporated into a vector for transformation into cells of the subject to be treated. Preferred embodiments of such vectors are described herein. The vector may be designed so as to integrate into the chromosomes of the subject, *e.g.*, retroviral vectors, or to replicate autonomously in the host cells. Vectors containing encoding α-L-iduronidase nucleotide sequences may be designed so as to provide for continuous or regulated expression of the enzyme. Additionally, the genetic vector encoding the enzyme may be designed so

as to stably integrate into the cell genome or to only be present transiently. The general methodology of conventional genetic therapy may be applied to polynucleotide sequences encoding α-L-iduronidase. Reviews of conventional genetic therapy techniques can be found in Friedman, *Science* 244:1275-1281 (1989); Ledley, J. *Inherit. Metab. Dis.* 13:587-616 (1990); and Tolstoshev *et al., Curr Opinions Biotech.* 1:55-61 (1990).

A particularly preferred method of administering the recombinant enzyme is intravenously. A particularly preferred composition comprises recombinant α-L-iduronidase, normal saline, phosphate buffer to maintain the pH at about 5.8 and human albumin. These ingredients may be provided in the following amounts:

α-L-iduronidase	0.05-0.2 mg/mL or 12,500-50,000 units per mL
Sodium chloride solution	150 mM in an IV bag, 50-250 cc total volume
Sodium phosphate buffer	10-50 mM, pH 5.8
Human albumin	1 mg/mL

The invention having been described, the following examples are offered to illustrate the subject invention by way of illustration, not by way of limitation.

EXAMPLE 1

Producing Recombinant Iduronidase

Standard techniques such as those described by Sambrook *et al.* (1987) "Molecular Cloning: A Laboratory Manual", 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. may be used to clone cDNA encoding human α-L-iduronidase. The human α-L-iduronidase cDNA previously cloned was subcloned into PRCCMV (InVitrogen) as a HindIII-XbaI fragment from a bluescript KS subclone. An intron cassette derived from the murine immunoglobulin Cot intron between exons 2 and 3 was constructed using PCR amplification of bases 788-1372 (Tucker *et al., Proc. Natl. Acad. Sci. USA* 78: 7684-7688 (1991) of clone pRIR14.5 (Kakkis *et al., Nucleic Acids Res.* 16:7796 (1988)). The cassette included 136 bp of the 3' end of exon 2 and 242 bp of the 5' end of exon 3, which would remain in the properly spliced cDNA. No ATG sequences are present in the coding, region of the intron cassette. The intron cassette was cloned into the HindIII site 5' of the α-L-iduronidase cDNA. The neo gene was deleted by digestion with Xhol followed by recircularizing the vector to make pCMVhldu.

One vial of the master cell bank is thawed and placed in three T150 flasks in DME/F12 plus supplements plus 10% FBS and 500 11g/ml G418. After 3-4 days, the cells are passaged using trypsin-EDTA to 6 high capacity roller bottles in the same medium. The innoculum of 2 x 10^9 cells is

added to a Wheaton microcarrier flask containing 60 grams of Cytodex 2 microcarriers, and DME/F12 plus supplements, 10% FBS and 500 11g/ml of G418 at a final volume of 13 liters. The flask is stirred by a Bellco overhead drive with a Perfusion wand stirrer. The culture is monitored by temperature, DO and pH probes and controlled using the Wheaton mini-pilot plant control system with a PC interface (BioPro software). The parameters are controlled at the set points, 37° C, 80% air saturation, and pH 6.7, using a heating- blanket, oxygen sparger and base pump. The culture is incubated for 3-4 days at which time the culture is coming out of log phase growth at 1-3 x 10^6 cells per ml. Thereafter, at 12 hour intervals, the medium is changed with PF-CHO medium (with custom modifications, JRH Biosciences). The first 2 collections are set aside as "washout". The third collection is the beginning of the production run. Sodium butyrate at final 2 mM is added every 48 hours to induce an increase in iduronidase expression. Production continues with medium changes of 10 liters every 12 hours and the collections filtered through a 1 micron filter to eliminate free cells and debris. The culture is monitored for temperature, pH and DO on a continuous basis. Twice daily, the culture is sampled before the medium change and assayed for cell condition and microorganisms by phase contrast microscopy, glucose content using a portable glucometer, iduronidase activity using a fluorescent substrate assay. Cell mass is assayed several times during the run using a total cellular protein assay. By the middle of the run, cell mass reaches 10^7 cells per ml. Collected production medium containing iduronidase is then concentrated five fold using an A/G Technology hollow fiber molecular filter with a 30,000 molecular weight cutoff. The concentrate is then diafiltrated with a minimum three fold volume of 0.2 M NaCl in 10 mM $NaP0_4$, pH 5. 8 over a period of 8 hours. This step removes Pluronics F68 and uronic acids from the concentrate. These molecules can inhibit function of the Heparin column. The concentrate is adjusted to pH 5.0, filtered through 1.0 and 0.2 micron filters and then loaded on a Heparin-Sepharose CL-6B column. The column is washed with 10 column volumes of 0.2 M NaCl, 10 mM $NaPO_4$, pH 5.3), and the enzyme eluted with 0.6 MI, 10mM$NaPO_4$,pH 5.8. The eluate is adjusted to 1.5 M NaCl, filtered through a 1 micron filter and loaded on a Phenyl-Sepharose HP column. The column is washed with 10 column volumes of 1.5 M NaCl, 10 mM $NaPO_4$, pH 5.8 and the enzyme eluted with 0. 15 M NaCl, 10 mM $NaPO_4$, pH 5.8.

Viral inactivation is performed by acidifying the enzyme fraction to pH 3.3 using 1 M citric acid pH 2.9 and incubating the enzyme at pH 3.3 at room temperature for 4 hours and readjusting the pH to 5.8 using 1 M phosphate buffer. This step has been demonstrated to remove 5 logs or better of a retrovirus in spiking experiments. The inactivated enzyme is filtered through a 0.2 μ filter, concentrated on an A/G Technologies hollow fiber concentrator apparatus (30,000 molecular weight cutoff) and injected in cycles on a Sephacryl S200 gel filtration column and the peaks collected. The pooled peaks are filtered through a 0.2 μ filter, formulated to 0.1 M $NaPO_4$, pH 5.8 and vialed.

A set of studies may be performed to assess the quality, purity, potency of the enzyme. Results of an SDS-PAGE analysis of the eluate is provided in Figure 2.

One recombinant human α-L-iduronidase obtained from this procedure demonstrates a potency of 100,000 units per milliliter and has a total protein concentration of 0.313 mg/ml.

EXAMPLE 2

Recombinant α-L-Iduronidase Therapy is Efficacious

Short-term intravenous administration of purified human recombinant α-L-iduronidase to 9 MPS I dogs and 6 MPS I cats has shown significant uptake of enzyme in a variety of tissues with an estimated 50% or more recovery in tissues 24 hours after a single dose. Although liver and spleen take up the largest amount of enzyme, and have the best pathologic improvement, improvements in pathology and glycosaminoglycan content has been observed in many, but not all tissues. In particular, the cartilage, brain and heart valve did not have significant improvement. Clinical improvement was observed in a single dog on long-term treatment for 13 months, but other studies have been limited to 6 months or less. All dogs, and most cats, that received recombinant human enzyme developed antibodies to the human product. The IgG antibodies are of the complement activating type (probable canine IgG equivalent). This phenomena is also observed in at least 13% of alglucerase-treated Gaucher patients. Proteinuria has been observed in one dog which may be related to immune complex disease. No other effects of the antibodies have been observed in the other treated animals. Specific toxicity was not observed and clinical laboratory studies (complete blood counts, electrolytes, BLJN/creatinine, liver enzymes, urinalysis) have been otherwise normal.

Enzyme therapy at even small doses of 25,000 units (0.1 mg/kg/wk) resulted in significant enzyme distribution to some tissues and decreases in GAG storage. If continued for over 1 year, significant clinical effects of the therapy were evident in terms of activity, mobility, growth and overall health. The therapy at this dose did not improve other tissues that are important sites for disease in this entity such as cartilage and brain. Higher doses of 125,000 units (0.5 mg/kg) given 5 times over two weeks demonstrate that improved tissue penetration can be achieved, and a therapeutic effect at the tissue level was accomplished in as little as 2 weeks. Studies at this increased dose are ongoing in two dogs for six months to date. These MPS I dogs showed significant clinical improvement and substantial decreases in urinary GAG excretion into the normal range. Other than an immune reaction controlled by altered administration techniques, the enzyme therapy has not shown significant clinical or biochemical toxicity. Enzyme therapy at this higher weekly dose is effective at improving some clinical features of MPS I and decreasing storage without significant toxicity.

The results of these various studies in MPS I dogs and one study in MPS I cats shows that human recombinant α-L-iduronidase is safe. These same results also provide a significant rationale that this recombinant enzyme should be effective in treating α-L-iduronidase deficiency.

EXAMPLE 3

Recombinant α-L-Iduronidase Therapy in Efficacious in Humans

The human cDNA of α-L-iduronidase predicts a protein of 653 amino acids and an expected molecular weight of 70,000 daltons after signal peptide cleavage. Amino acid sequencing reveals alanine 26 at the N-terminus giving an expected protein of 629 amino acids. Human recombinant α-L-iduronidase has a Histidine at position 8 of the mature protein. The predicted protein sequence comprises six potential N-linked oligosaccharide modification sites. All of these sites are modified in the recombinant protein. The third and sixth sites have been demonstrated to contain one or more mannose 6-phosphate residues responsible for high affinity uptake into cells.

This peptide corresponds to Amino Acids 26-45 of Human Recombinant α-L-iduronidase with an N-terminus alanine and the following sequence:

ala-glu-ala-pro-his-leu-val-his-val-asp-ala-ala-arg-ala-leu-trp-pro-leu-arg-arg

The recombinant enzyme has an apparent molecular weight of 82,000 daltons on SDS-PAGE due to carbohydrate modifications. Purified human recombinant α-L-iduronidase has been sequenced by the UCLA Protein Sequencing facility. It is preferred to administer the recombinant enzyme intravenously. Human recombinant α-L-iduronidase was supplied in 10 mL polypropylene vials at a concentration of 0.05-0.2 mg/ml (12,500-50,000 units per mL). The final dosage form of the enzyme includes human recombinant α-L-iduronidase, normal saline, phosphate buffer at pH 5.8 and human albumin at 1 mg/ml. These are prepared in a bag of normal saline.

Component	**Composition**
α-L-iduronidase	0.05-0.2 mg/mL or 12,500-50,000 units per mL
Sodium chloride solution	150 mM in an IV bag, 50-250 cc total volume
Sodium phosphate buffer	10-50 mM, pH 5.8
Human albumin	1 mg/mL

Human patients manifesting a clinical phenotype of MPS-I disorder with an α-L-iduronidase level of less than 1% of normal in leukocytes and fibroblasts were included in the study. All patients manifested some clinical evidence of visceral and soft tissue accumulation of glycosaminoglycans with varying degrees of functional impairment. Efficacy was determined by measuring the

percentage reduction in urinary GAG excretion over time. FIGURE 3 reveals the urinary GAG levels in 16 MPS-I patients in relation to normal excretion values. There is a wide range of urine GAG values in untreated MPS-I patients. A greater than 50% reduction in excretion of undegraded GAGs following therapy with recombinant α-L-iduronidase is a valid means to measure an individual's response to therapy. FIGURE 4 demonstrates leukocyte iduronidase activity before and after enzyme therapy in MPS I patients. The buccal iduronidase activity before and after enzyme therapy is depicted in FIGURE 5. FIGURE 6 demonstrates in three patients that a substantial shrinkage of liver and spleen together with significant clinical improvement in joint and soft tissue storage was associated with a greater than 65% reduction in undegraded GAG after only 8 weeks of treatment with recombinant enzyme. FIGURE 7 demonstrates that there is substantial normalization of livers and spleens in patients treated with recombinant enzyme after only 12 weeks of therapy with recombinant enzyme. FIGURE 8 demonstrates a precipitous drop in urinary GAG excretion over 22 weeks of therapy with recombinant enzyme in 11 patients. Clinical assessment of liver and spleen size has been the most widely accepted means for evaluating successful bone marrow transplant treatment in MPS-I patients (Hoogerbrugge *et al.*, *Lancet* 345:1398 (1995)). Such measurements are highly correlated with a decreased visceral storage of GAGs in MPS-I patients.

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

WHAT IS CLAIMED:

1. A method for producing α-L-iduronidase comprising the step of transforming a suitable cell line with a cDNA encoding for all of α-L-iduronidase or a biologically active fragment or mutant thereof.

2. The method of claim 1 wherein the suitable cell line is a Chinese hamster ovary cell line 2.131.

3. The method according to claim 2 wherein the Chinese hamster cell line secretes about 5,000 to 7,000 fold more α-L-iduronidase than it secretes before introducing the cDNA encoding for all of the α-L-iduronidase or a biologically active fragment thereof.

4. The method according to claim 1 wherein the transfected cells are grown on microcarriers.

5. The method according to claim 1 wherein a culture system is optimized such that the culture pH is lowered to about 6.7-6.8 during the production process.

6. The method according to claim 1 wherein about 2/3 to 3/4 of a culture system growth medium is changed approximately every 12 hours.

7. The method according to claim 1 wherein a culture system oxygen saturation is optimized at about 80%.

8. The method of claim 7 wherein the culture system oxygen saturation is optimized at about 80% using intermittent pure oxygen sparging.

9. The method of claim 1 wherein microcarriers having about 10% serum initially are used to produce a cell mass for a culture system.

10. The method of claim 1 further comprising the step of a washout shift to protein-free medium for production.

11. The method of claim 1 wherein a culture system comprising a JRH Biosciences PF-CHO growth medium is used.

12. The method of claim 11 wherein said growth medium is optimized to include supplemental amounts of one or more ingredients selected from the group consisting of glutamate, aspartate, glycine, ribonucleosides and deoxyribonucleosides.

13. The method according to claim 1 wherein a batch-feed process is performed by a perfusion wand.

14. The method according to claim 1 wherein sodium butyrate is added to a culture system.

15. A transfected cell line having the ability to produce α-L-iduronidase.

16. A transfected cell line according to claim 15 wherein the transfected cell line is a recombinant Chinese Hamster ovary cell line.

17. A transfected cell line according to claim 15 wherein the transfected cell line is a recombinant Chinese hamster ovary 2.131 cell line.

18. A transfected cell line according to claim 15 wherein the transfected cell line contains at least about 10 copies of an expression construct comprising a CMV promoter, a Ca intron, an α-L-iduronidase cDNA, and a bovine growth hormone polyadenylation sequence.

19. A transfected cell line according to claim 15 wherein the transfected cell line expresses α-L-iduronidase at amounts of at least about 20-40 micrograms per 10^7 cells per day.

20. A vector adapted to produce human α-L-iduronidase in a transfected cell.

21. The vector according to claim 20 adapted to produce human α-L-iduronidase in a Chinese hamster ovary (CHO) cell.

22. The vector according to claim 20 comprising a CMV immediate early gene promoter/enhancer.

23. The vector according to claim 20 comprising a cytomegalovirus promoter/enhancer element, a 5' intron consisting of a murine Ca intron between exons 2 and 3, a cDNA encoding for all or a biologically active fragment of a α-L-iduronidase and a 3' bovine growth hormone polyadenylation site.

24. A recombinant α-L-iduronidase produced in accordance with the method of claim 1.

25. An α-L-iduronidase produced according to the method of claim 1 having a specific activity of at least about 200,000 units per milligram.

26. An α-L-iduronidase according to claim 25 having a specific activity of at least about 240,000 units per milligram.

27. A method of purifying α-L-iduronidase comprising the steps of:

(a) performing a concentration/diafiltration procedure to remove one or more undesirable compounds from a sample;

(b) acidifying the sample of step (a);

(c) running the sample of step (b) on a heparin column;

(d) running the sample of step (c) on a phenyl column;

(e) running the sample of step (d) on a Sephacryl column; and

(f) running the substantially purified α-L-iduronidase.

28. A method of treating a disease caused all or in part by a deficiency in α-L-iduronidase comprising the step of administering a recombinant α-L-iduronidase.

29. A method for treating a disease in a human caused all or in part by a deficiency in α-L-iduronidase comprising the step of administering a recombinant human α-L-iduronidase.

30. The method of claim 28 wherein the disease is mucopolysaccharidosis.

31. The method of claim 28 wherein the disease is MPS I.

32. The method of claim 28 wherein the disease is selected from the group consisting of Hurler's disease, Scheie syndrome and Hurler-Scheie syndrome.

33. The method of claim 28 wherein a patient suffering from the disease demonstrates about 1% or less of a normal α-L-iduronidase activity.

34. The method of claim 28 wherein at least about 25,000 units or 0.1 mg/kg of a recombinant α-L-iduronidase are administered weekly to a patient suffering from a deficiency thereof.

35. The method of claim 28 wherein at least about 125,000 units or 0.5mg/kg of a recombinant α-L-iduronidase are administered weekly to a patient suffering from a deficiency thereof.

36. A pharmaceutical composition comprising recombinant α-L-iduronidase and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36 further comprising a sodium chloride solution, a buffer and human albumin.

38. The pharmaceutical composition of claim 36 wherein the recombinant α-L-iduronidase is present at a concentration of about 0.05 to 0.20 mg/mL or about 12,500 to about 50,000 units per mL.

39. The pharmaceutical composition of claim 36 wherein the human albumin is present at a concentration of at least about 1 mg/mL.

40. The pharmaceutical composition of claim 36 wherein the buffer is a sodium phosphate buffer at a concentration of about 10-50 mM.

41. The pharmaceutical composition of claim 36 wherein the pH of the composition is maintained at about 5.8.

FIG. 1-1

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        10         20         30         40         50         60         70
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT
        80         90        100        110        120        130        140
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG CGAGCAAAAT TTAAGCTACA
       150        160        170        180        190        200        210
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG
       220        230        240        250        260        270        280
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ATGTACGGGC CAGATATACG CGTTGACATT GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC
       290        300        310        320        330        340        350
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ATTAGTTCAT AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
       360        370        380        390        400        410        420
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC
       430        440        450        460        470        480        490
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC
       500        510        520        530        540        550        560
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA
       570        580        590        600        610        620        630
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC
       640        650        660        670        680        690        700
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
       710        720        730        740        750        760        770
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG
       780        790        800        810        820        830        840
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
       850        860        870        880        890        900        910
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCCA AGCTTCGCAG AATTCCTGCG
       920        930        940        950        960        970        980
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
GCTGCTACAG TGTGTCCAGC GTCCTGCCTG GCTGTGCTGA GCGCTGGAAC AGTGGCGCAT CATTCAAGTG
       990       1000       1010       1020       1030       1040       1050
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
CACAGTTACC CATCCTGAGT CTGGCACCTT AACTGGCACA ATTGCCAAAG TCACAGGTGA GCTCAGATGC
      1060       1070       1080       1090       1100       1110       1120
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ATACCAGGAC ATTGTATGAC GTTCCCTGCT CACATGCCTG CTTTCTTCCT ATAATACAGA TGCTCAACTA
      1130       1140       1150       1160       1170       1180       1190
     *   *      *   *      *   *      *   *      *   *      *   *      *   *
ACTGCTCATG TCCTTATATC ACAGAGGGAA ATTGGAGCTA TCTGAGGAAC TGCCCAGAAG GGAAGGGCAG
```

FIG. 1-2

```
        1200      1210      1220      1230      1240      1250      1260
      *    *    *    *    *    *    *    *    *    *    *    *    *    *
AGGGGTCTTG CTCTCCTTGT CTGAGCCATA ACTCTTCTTT CTACCTTCCA GTGAACACCT TCCCACCCCA
        1270      1280      1290      1300      1310      1320      1330
      *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGTCCACCTG CTACCGCCGC CGTCGGAGGA GCTGGCCCTG AATGAGCTCT TGTCCCTGAC ATGCCTGGTG
        1340      1350      1360      1370      1380      1390      1400
      *    *    *    *    *    *    *    *    *    *    *    *    *    *
CGAGCTTTCA ACCCTAAAGA AGTGCTGGTG CGATGGCTGC ATGGAAATGA GGAGCTGTCC CCAGAAAGCT
        1410      1420      1430      1440      1450      1460      1470
      *    *    *    *    *    *    *    *    *    *    *    *    *    *
ACCTAGTGTT TGAGCCCCTA AAGGAGCCAG GCGAGGGAGC CACCACCTAC CTGGTGACAA GCGTGTTGCG
        1480      1490      1500      1510      1520      1530      1540
      *    *    *    *    *    *    *    *    *    *    *    *    *    *
TGTATCAGCT GAAAGCTTGA TATCGAATTC CGGAGGCGGA ACCGGCAGTG CAGCCCGAAG CCCCGCAGTC
        1550      1560       1570       1580       1590
      *    *    *    *     *    *    *    *    *    *    *
CCCGAGCACG CGTGGCC ATG CGT CCC CTG CGC CCC CGC GCC GCG CTG CTG GCG CTC CTG
                   Met Arg Pro Leu Arg Pro Arg Ala Ala Leu Leu Ala Leu Leu
1600       1610       1620       1630       1640       1650
  *    *    *    *    *    *    *    *    *    *    *    *
GCC TCG CTC CTG GCC GCG CCC CCG GTG GCC CCG GCC GAG GCC CCG CAC CTG GTG CAT
Ala Ser Leu Leu Ala Ala Pro Pro Val Ala Pro Ala Glu Ala Pro His Leu Val His
  1660       1670       1680       1690       1700       1710
      *    *    *    *    *    *    *    *    *    *    *
GTG GAC GCG GCC CGC GCG CTG TGG CCC CTG CGG CGC TTC TGG AGG AGC ACA GGC TTC
Val Asp Ala Ala Arg Ala Leu Trp Pro Leu Arg Arg Phe Trp Arg Ser Thr Gly Phe
     1720       1730       1740       1750       1760       1770
  *    *    *    *    *    *    *    *    *    *    *    *
TGC CCC CCG CTG CCA CAC AGC CAG GCT GAC CAG TAC GTC CTC AGC TGG GAC CAG CAG
Cys Pro Pro Leu Pro His Ser Gln Ala Asp Gln Tyr Val Leu Ser Trp Asp Gln Gln
        1780       1790       1800       1810       1820
      *    *    *    *    *    *    *    *    *    *    *
CTC AAC CTC GCC TAT GTG GGC GCC GTC CCT CAC CGC GGC ATC AAG CAG GTC CGG ACC
Leu Asn Leu Ala Tyr Val Gly Ala Val Pro His Arg Gly Ile Lys Gln Val Arg Thr
1830       1840       1850       1860       1870       1880
  *    *    *    *    *    *    *    *    *    *    *
CAC TGG CTG CTG GAG CTT GTC ACC ACC AGG GGG TCC ACT GGA CGG GGC CTG AGC TAC
His Trp Leu Leu Glu Leu Val Thr Thr Arg Gly Ser Thr Gly Arg Gly Leu Ser Tyr
    1890       1900       1910       1920       1930       1940
  *    *    *    *    *    *    *    *    *    *    *    *
AAC TTC ACC CAC CTG GAC GGG TAC CTG GAC CTT CTC AGG GAG AAC CAG CTC CTC CCA
Asn Phe Thr His Leu Asp Gly Tyr Leu Asp Leu Leu Arg Glu Asn Gln Leu Leu Pro
       1950       1960       1970       1980       1990
      *    *    *    *    *    *    *    *    *    *    *
GGG TTT GAG CTG ATG GGC AGC GCC TCG GGC CAC TTC ACT GAC TTT GAG GAC AAG CAG
Gly Phe Glu Leu Met Gly Ser Ala Ser Gly His Phe Thr Asp Phe Glu Asp Lys Gln
```

FIG. 1-3

2000 2010 2020 2030 2040 2050
CAG GTG TTT GAG TGG AAG GAC TTG GTC TCC AGC CTG GCC AGG AGA TAC ATC GGT AGG
Gln Val Phe Glu Trp Lys Asp Leu Val Ser Ser Leu Ala Arg Arg Tyr Ile Gly Arg

2060 2070 2080 2090 2100 2110
TAC GGA CTG GCG CAT GTT TCC AAG TGG AAC TTC GAG ACG TGG AAT GAG CCA GAC CAC
Tyr Gly Leu Ala His Val Ser Lys Trp Asn Phe Glu Thr Trp Asn Glu Pro Asp His

2120 2130 2140 2150 2160
CAC GAC TTT GAC AAC GTC TCC ATG ACC ATG CAA GGC TTC CTG AAC TAC TAC GAT GCC
His Asp Phe Asp Asn Val Ser Met Thr Met Gln Gly Phe Leu Asn Tyr Tyr Asp Ala

2170 2180 2190 2200 2210 2220
TGC TCG GAG GGT CTG CGC GCC GCC AGC CCC GCC CTG CGG CTG GGA GGC CCC GGC GAC
Cys Ser Glu Gly Leu Arg Ala Ala Ser Pro Ala Leu Arg Leu Gly Gly Pro Gly Asp

2230 2240 2250 2260 2270 2280
TCC TTC CAC ACC CCA CCG CGA TCC CCG CTG AGC TGG GGC CTC CTG CGC CAC TGC CAC
Ser Phe His Thr Pro Pro Arg Ser Pro Leu Ser Trp Gly Leu Leu Arg His Cys His

2290 2300 2310 2320 2330 2340
GAC GGT ACC AAC TTC TTC ACT GGG GAG GCG GGC GTG CGG CTG GAC TAC ATC TCC CTC
Asp Gly Thr Asn Phe Phe Thr Gly Glu Ala Gly Val Arg Leu Asp Tyr Ile Ser Leu

2350 2360 2370 2380 2390
CAC AGG AAG GGT GCG CGC AGC TCC ATC TCC ATC CTG GAG CAG GAG AAG GTC GTC GCG
His Arg Lys Gly Ala Arg Ser Ser Ile Ser Ile Leu Glu Gln Glu Lys Val Val Ala

2400 2410 2420 2430 2440 2450
CAG CAG ATC CGG CAG CTC TTC CCC AAG TTC GCG GAC ACC CCC ATT TAC AAC GAC GAG
Gln Gln Ile Arg Gln Leu Phe Pro Lys Phe Ala Asp Thr Pro Ile Tyr Asn Asp Glu

2460 2470 2480 2490 2500 2510
GCG GAC CCG CTG GTG GGC TGG TCC CTG CCA CAG CCG TGG AGG GCG GAC GTG ACC TAC
Ala Asp Pro Leu Val Gly Trp Ser Leu Pro Gln Pro Trp Arg Ala Asp Val Thr Tyr

2520 2530 2540 2550 2560
GCG GCC ATG GTG GTG AAG GTC ATC GCG CAG CAT CAG AAC CTG CTA CTG GCC AAC ACC
Ala Ala Met Val Val Lys Val Ile Ala Gln His Gln Asn Leu Leu Leu Ala Asn Thr

2570 2580 2590 2600 2610 2620
ACC TCC GCC TTC CCC TAC GCG CTC CTG AGC AAC GAC AAT GCC TTC CTG AGC TAC CAC
Thr Ser Ala Phe Pro Tyr Ala Leu Leu Ser Asn Asp Asn Ala Phe Leu Ser Tyr His

2630 2640 2650 2660 2670 2680
CCG CAC CCC TTC GCG CAG CGC ACG CTC ACC GCG CGC TTC CAG GTC AAC AAC ACC CGC
Pro His Pro Phe Ala Gln Arg Thr Leu Thr Ala Arg Phe Gln Val Asn Asn Thr Arg

FIG. 1-4

```
       2690         2700          2710          2720          2730
CCG CCG CAC GTG CAG CTG TTG CGC AAG CCG GTG CTC ACG GCC ATG GGG CTG CTG GCG
Pro Pro His Val Gln Leu Leu Arg Lys Pro Val Leu Thr Ala Met Gly Leu Leu Ala
2740      2750          2760          2770          2780          2790
CTG CTG GAT GAG GAG CAG CTC TGG GCC GAA GTG TCG CAG GCC GGG ACC GTC CTG GAC
Leu Leu Asp Glu Glu Gln Leu Trp Ala Glu Val Ser Gln Ala Gly Thr Val Leu Asp
 2800          2810          2820          2830          2840          2850
AGC AAC CAC ACG GTG GGC GTC CTG GCC AGC GCC CAC CGC CCC CAG GGC CCG GCC GAC
Ser Asn His Thr Val Gly Val Leu Ala Ser Ala His Arg Pro Gln Gly Pro Ala Asp
    2860          2870          2880          2890          2900          2910
GCC TGG CGC GCC GCG GTG CTG ATC TAC GCG AGC GAC GAC ACC CGC GCC CAC CCC AAC
Ala Trp Arg Ala Ala Val Leu Ile Tyr Ala Ser Asp Asp Thr Arg Ala His Pro Asn
       2920          2930          2940          2950          2960
CGC AGC GTC GCG GTG ACC CTG CGG CTG CGC GGG GTG CCC CCC GGC CCG GGC CTG GTC
Arg Ser Val Ala Val Thr Leu Arg Leu Arg Gly Val Pro Pro Gly Pro Gly Leu Val
2970          2980          2990          3000          3010          3020
TAC GTC ACG CGC TAC CTG GAC AAC GGG CTC TGC AGC CCC GAC GGC GAG TGG CGG CGC
Tyr Val Thr Arg Tyr Leu Asp Asn Gly Leu Cys Ser Pro Asp Gly Glu Trp Arg Arg
   3030          3040          3050          3060          3070          3080
CTG GGC CGG CCC GTC TTC CCC ACG GCA GAG CAG TTC CGG CGC ATG CGC GCG GCT GAG
Leu Gly Arg Pro Val Phe Pro Thr Ala Glu Gln Phe Arg Arg Met Arg Ala Ala Glu
       3090          3100          3110          3120          3130
GAC CCG GTG GCC GCG GCG CCC CGC CCC TTA CCC GCC GGC GGC CGC CTG ACC CTG CGC
Asp Pro Val Ala Ala Ala Pro Arg Pro Leu Pro Ala Gly Gly Arg Leu Thr Leu Arg
3140          3150          3160          3170          3180          3190
CCC GCG CTG CGG CTG CCG TCG CTT TTG CTG GTG CAC GTG TGT GCG CGC CCC GAG AAG
Pro Ala Leu Arg Leu Pro Ser Leu Leu Leu Val His Val Cys Ala Arg Pro Glu Lys
   3200          3210          3220          3230          3240          3250
CCG CCC GGG CAG GTC ACG CGG CTC CGC GCC CTG CCC CTG ACC CAA GGG CAG CTG GTT
Pro Pro Gly Gln Val Thr Arg Leu Arg Ala Leu Pro Leu Thr Gln Gly Gln Leu Val
       3260          3270          3280          3290          3300
CTG GTC TGG TCG GAT GAA CAC GTG GGC TCC AAG TGC CTG TGG ACA TAC GAG ATC CAG
Leu Val Trp Ser Asp Glu His Val Gly Ser Lys Cys Leu Trp Thr Tyr Glu Ile Gln
3310          3320          3330          3340          3350          3360
TTC TCT CAG GAC GGT AAG GCG TAC ACC CCG GTC AGC AGG AAG CCA TCG ACC TTC AAC
Phe Ser Gln Asp Gly Lys Ala Tyr Thr Pro Val Ser Arg Lys Pro Ser Thr Phe Asn
```

FIG. 1-5

```
3370       3380       3390       3400       3410       3420
  *    *     *     *    *     *     *    *     *     *     *
CTC TTT GTG TTC AGC CCA GAC ACA GGT GCT GTC TCT GGC TCC TAC CGA GTT CGA GCC
Leu Phe Val Phe Ser Pro Asp Thr Gly Ala Val Ser Gly Ser Tyr Arg Val Arg Ala
    3430       3440       3450       3460       3470       3480
*    *     *     *    *     *     *    *     *     *     *
CTG GAC TAC TGG GCC CGA CCA GGC CCC TTC TCG GAC CCT GTG CCG TAC CTG GAG GTC
Leu Asp Tyr Trp Ala Arg Pro Gly Pro Phe Ser Asp Pro Val Pro Tyr Leu Glu Val
        3490       3500       3510       3520       3530       3540
   *     *     *     *    *     *     *    *     *     *     *
CCT GTG CCA AGA GGG CCC CCA TCC CCG GGC AAT CCA TGAG CCTGTGCTGA GCCCCAGTGG
Pro Val Pro Arg Gly Pro Pro Ser Pro Gly Asn Pro
      3550       3560       3570       3580       3590       3600       3610
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
GTTGCACCTC CACCGGCAGT CAGCGAGCTG GGGCTGCACT GTGCCCATGC TGCCCTCCCA TCACCCCCTT
      3620       3630       3640       3650       3660       3670       3680
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
TGCAATATAT TTTTATATTT TAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
      3690       3700       3710       3720       3730       3740       3750
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
AAAAAAAAAA AAAAAAAAAG AATTCCTGCA GCCCGGGGGA TCCACTAGTT CTAGAGGGCC CGTTTAAACC
      3760       3770       3780       3790       3800       3810       3820
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT
      3830       3840       3850       3860       3870       3880       3890
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG
      3900       3910       3920       3930       3940       3950       3960
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA AGACAATAGC
      3970       3980       3990       4000       4010       4020       4030
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC CAGCTGGGGC TCGAGAGCTT
      4040       4050       4060       4070       4080       4090       4100
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
GGCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA
      4110       4120       4130       4140       4150       4160       4170
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT
      4180       4190       4200       4210       4220       4230       4240
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG
      4250       4260       4270       4280       4290       4300       4310
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG
      4320       4330       4340       4350       4360       4370       4380
   *    *     *    *     *    *     *    *     *    *     *    *     *    *
CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA
```

FIG. 1-6

```
      4390      4400      4410      4420      4430      4440      4450
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA
      4460      4470      4480      4490      4500      4510      4520
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA
      4530      4540      4550      4560      4570      4580      4590
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA
      4600      4610      4620      4630      4640      4650      4660
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCACGCT GTAGGTATCT
      4670      4680      4690      4700      4710      4720      4730
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC
      4740      4750      4760      4770      4780      4790      4800
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA
      4810      4820      4830      4840      4850      4860      4870
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA
      4880      4890      4900      4910      4920      4930      4940
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT
      4950      4960      4970      4980      4990      5000      5010
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA
      5020      5030      5040      5050      5060      5070      5080
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA
      5090      5100      5110      5120      5130      5140      5150
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA
      5160      5170      5180      5190      5200      5210      5220
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA
      5230      5240      5250      5260      5270      5280      5290
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT
      5300      5310      5320      5330      5340      5350      5360
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG
      5370      5380      5390      5400      5410      5420      5430
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT
      5440      5450      5460      5470      5480      5490      5500
   *    *    *    *    *    *    *    *    *    *    *    *    *    *
CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG
```

FIG. 1-7

```
      5510       5520       5530       5540       5550       5560       5570
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC
      5580       5590       5600       5610       5620       5630       5640
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC
      5650       5660       5670       5680       5690       5700       5710
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC
      5720       5730       5740       5750       5760       5770       5780
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA
      5790       5800       5810       5820       5830       5840       5850
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA
      5860       5870       5880       5890       5900       5910       5920
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG
      5930       5940       5950       5960       5970       5980       5990
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT
      6000       6010       6020       6030       6040       6050       6060
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC
      6070       6080       6090       6100       6110       6120       6130
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT
      6140       6150       6160       6170       6180       6190       6200
         *    *     *    *     *    *     *    *     *    *     *    *     *    *
TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
```

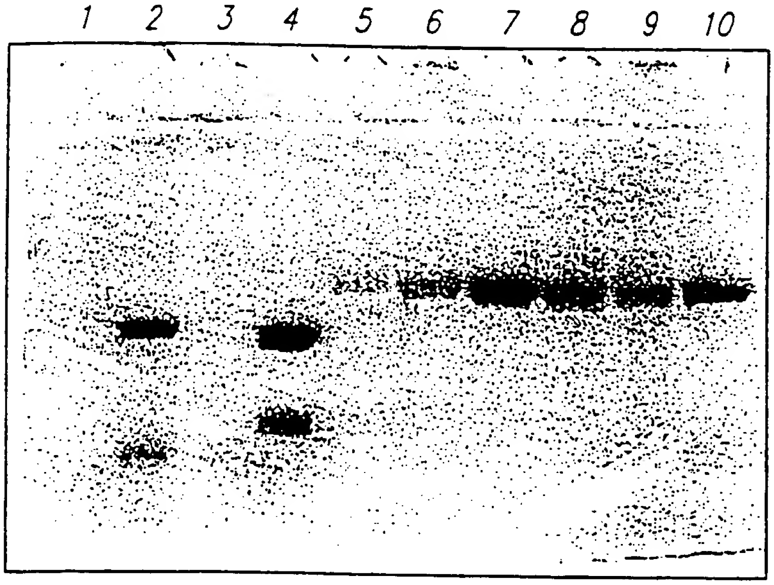

FIG. 2

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(54) Title: RECOMBINANT (ALPHA)-L-IDURONIDASE, METHODS FOR PRODUCING AND PURIFYING THE SAME AND METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES THEREOF

(57) Abstract

The present invention provides a recombinant α-L-iduronidase and biologically active fragments and mutants thereof, methods to produce and purify this enzyme as well as methods to treat certain genetic disorders including α-L-iduronidase deficiency and mucopolysaccharidosis I (MPS I).

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/10102

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/56 C12N15/85 C12N9/24 C12N5/00 C12N5/10
A61K38/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAKKIS E ET AL: "Overexpression of the human lysosomal enzyme alpha-L-iduronidase in Chinese hamster ovary cells" PROTEIN EXPR PURIF, vol. 5, no. 3, June 1994 (1994-06), pages 225-232, XP000857380	1-6,9, 15-26
Y	the whole document	28-41
X	UNGER E ET AL: "Recombinant alpha-L-iduronidase: characterization of the purified enzyme and correction of mucopolysaccharidosis type I fibroblasts" BIOCHEM J, vol. 304, 15 November 1994 (1994-11-15), pages 43-49, XP000857388	1,10,15, 16, 19-21, 24-26
Y	figures 8,9	28-41

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 December 1999	27/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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INTERNATIONAL SEARCH REPORT

Inter. onal Application No
PCT/US 99/10102

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
Y	SHULL R ET AL : "Enzyme replacement in a canine model of Hurler syndrome" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 91, no. 26, 20 December 1994 (1994-12-20), pages 12937-12941, XP002125064 page 12938, column 1, paragraph 3 ---	28-41
Y	KAKKIS E ET AL: "Long-term and high-dose trials of enzyme replacement therapy in the canine model of mucopolysaccharidosis I" BIOCHEM MOL MED, vol. 58, no. 2, August 1996 (1996-08), pages 156-167, XP000862844 page 157, column 2 -page 158, column 1 ---	28-41
X	WO 93 10244 A (WOMEN S AND CHILDREN S HOSPITA) 27 May 1993 (1993-05-27) page 17, paragraph 1; page 9, paragraph 2 ---	1,15,16, 20,21, 24,28-36
A	CLEMENTS P ET AL: "Human alpha-L-iduronidase. 1. Purification, monoclonal antibody production, native and subunit molecular mass" EUR J BIOCHEM, vol. 152, no. 1, 1 October 1985 (1985-10-01), pages 21-28, XP000857400 ---	
A	WO 97 10353 A (CROPTECH DEV CORP ;VIRGINIA TECH INTELL PROP (US)) 20 March 1997 (1997-03-20) -----	

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 28-35, as far as in vivo methods are concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26, 28-41 (all totally)

   A method for producing a-L-iduronidase comprising the step of transforming a suitable cell line with a cDNA encoding for all of a-L-iduronidase or a biologically active fragment or mutant thereof; a transfected cell line having the ability to produce a-L-iduronidase; a vector adapted to produce human a-L-iduronidase in a transfected cell; a recombinant a-L-iduronidase; an a-L-iduronidase having a specific activity of at least about 200000 units per milligram; a method of tretaing a disease comprising the step of administering a recombinant a-L-iduronidase; a pharmaceutical composition comprising recombinant a-L-iduronidase and a pharmaceutically acceptable carrier

2. Claim : 27 (totally)

   A method of purifying a-L-iduronidase comprising steps a) to f)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/10102

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9310244 A	27-05-1993	AU 649897 B	02-06-1994
		AU 2914192 A	15-06-1993
		CA 2099503 A	15-05-1993
		EP 0578790 A	19-01-1994
		JP 6504449 T	26-05-1994
		NZ 245123 A	26-05-1995
WO 9710353 A	20-03-1997	AU 7071196 A	01-04-1997
		EP 0865499 A	23-09-1998
		US 5929304 A	27-07-1999